Muscle-derived stem cells (MDSCs) have the capacity to regenerate bone and muscle in vitro (4, 7, 8). To refine therapeutic options for the use of MDSCs in regenerative medicine, the response of these progenitor cells should be tailored for each specific application. The purpose of this study was to engineer patterned cell differentiation of a single population of mouse MDSCs towards the osteogenic and myogenic lineages simultaneously in vitro in the same well. We based our strategy on the assumption that morphogens exert tissue-specific differentiative effects as solid-phase patterns in vivo (2, 3, 5, 9, 10) during development and regeneration of tissues.

Methods

To engineer cell response to solid-phase morphogen patterns, we used our established ink-jet printing technology (1, 6) to deposit patterned arrays of BMP-2. We created square arrays (750 µm x 750 µm) of solid-phase, immobilized patterns of BMP-2 on fibrin films and used Cy-3 labeled BMP-2 to visualize the printed patterns. Surface concentration of deposited Cy-3-BMP-2 was controlled using an overprinting strategy (Fig1). MDSCs were seeded on BMP-2 patterns to ~70% confluence and cultured in either 20% or 2% serum for 72 hrs. Induction of ALP activity was used to detect differentiation of MDSCs towards the osteogenic lineage on BMP-2 patterns. To demonstrate differentiation towards the myogenic lineage, immunocytochemical staining for myosin heavy chain which is expressed in fused myotubes was performed. To demonstrate further control over BMP-2 driven patterned osteogenic differentiation, fibroblast growth factor 2 (FGF-2) was co-printed with BMP-2 in square patterns using multiple concentrations and combinations to employ the ink-jet printing in a high-throughput approach.

Results

MDSC populations differentiate towards the osteogenic lineage in direct register to square patterns of bioprinted BMP-2 (Fig2C) in a dose-dependent manner (Fig2D-G), shown by induction of ALP activity. Negative (Fig2A) and positive controls (Fig2B) showed uniform cell response across the entire cell population. Cells off pattern do not express substantial ALP activity. FGF-2 and BMP-2 co-printed patterns reduced progression of osteogenic lineage on pattern in a dose-dependent manner. MDSCs cultured on BMP-2 patterns under serum deprivation resulted in differentiation towards the osteogenic lineage in register to BMP-2 printed patterns and towards the myogenic lineage off pattern (Fig3). Myogenic differentiation as evidenced by myotubes formation increased with distance from BMP-2 patterns.

Discussion

This work provides proof-of-concept for engineered patterned cell differentiation, and demonstrates an enabling high-throughput technology with which to elucidate the basic biological mechanisms by which MDSCs and other multi-potent cell populations differentiate towards multiple lineages. Researchers could use this approach to tailor tissue-engineered therapies for specific regenerative applications for multiple tissues.

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References


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